



IN THE UNITED STATES PATENT
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PATENT
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Applicants: Erik H.F. Wong et al.)
Serial No.: 09/599,213)
Filed: June 22, 2000)
Title: METHOD OF TREATING OR)
PREVENTING CHRONIC)
PAIN WITH A HIGHLY)
SELECTIVE)
NOREPINEPHRINE)
REUPTAKE INHIBITOR (*As*)
Amended))
Group Art Unit: 1614)
Examiner: William R.A. Jarvis)
Attorney Docket No.: 6248.4)

DECLARATION OF STEPHEN P. ARNERIC
PURSUANT TO 37 C.F.R. § 1.132

Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Stephen P. Arneric, hereby declare that:

1. I have reviewed the above-captioned patent application and am familiar with the subject matter disclosed therein.
2. I have reviewed and am familiar with a U.S. Patent and Trademark Office (USPTO) official action dated October 10, 2001, in which the USPTO commented on the application.
3. On February 19, 2002, I participated in an interview at the USPTO with attorneys prosecuting the application (James J. Napoli and Sandip H. Patel) and the examiner responsible for reviewing the application (William R.A. Jarvis).
4. In 1979, I received a B.S. degree in Physical Science from the Lyman Briggs College at Michigan State University (East Lansing, MI), and in 1983, I received a Ph.D. in Pharmacology from the University of Iowa (Iowa City, IA).

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5. From 1983 to 1985, I conducted post-doctoral research in the Department of Neurobiology/Cerebral Circulation at Cornell University Medical College (New York, NY). From 1985 to 1986, I was an Assistant Professor in the Department of Neurobiology at Cornell University Medical College.

From 1987 to February of 1998, I was employed by Abbott Laboratories (Abbott Park, IL), where I held the following positions: Research Investigator and Project/Group Leader for Cognitive Function (1989 to 1991); Project Leader for Cholinergic Channel Modulators in the Neuroscience Pharmaceutical Discovery Division (1991 to 1997); and, Director of Neurological and Urological Diseases Research in the Neuroscience Pharmaceutical Discovery Division (1997 to 1998).

From March of 1998 to May of 2000, I was a Senior Director in Central Nervous System (CNS) Research at DuPont Pharmaceuticals Company (Wilmington, DE).

Since May of 2000, I have been employed by Pharmacia Corporation (Kalamazoo, MI) as a Director in Neurobiology and Central Nervous System (CNS) Discovery Research.

Additionally, since 1986, I have been employed by Southern Illinois University School of Medicine (Springfield, IL), where I have held the following positions: Assistant Professor (1986 to 1989); Adjunct Assistant Professor (1989 to 1992); Adjunct Associate Professor (1992 to 1996); and, Adjunct Professor (1996 to present).

6. Based on my academic and employment experiences, I am qualified to provide the comments set forth herein regarding the subject matter of the above-captioned application.
7. I have reviewed and am familiar with Max *et al.* (1991) *Pain* 45:3-10 (hereafter "the Max 1991 article").
8. Attached hereto are Tables I and II, which respectively set forth inhibition constants of compounds for various monoamine transporters and receptors, and the selectivity for the norepinephrine transporter over the serotonin transporter. The selectivity values reported in the last column of Table II are obtained by dividing the inhibition constant (K_i , nM) for serotonin by the inhibition constant (K_i , nM) for

norepinephrine. The selectivity value is a unitless number, where a value equal to one represents no selectivity (i.e., equal affinity for both transporters), values greater than one represent greater norepinephrine selectivity, and values less than one represent greater serotonin selectivity.

9. As reported in Table II, desipramine exhibits selectivity (430 fold) for the norepinephrine transporter site over that of the serotonin transporter site. In the 1991 Max article, Max et al. have suggested that blockade of norepinephrine reuptake, an action shared by desipramine, amitriptyline, and other antidepressants proven effective in neuropathic pain, may mediate the pain relief. In their study, Max et al. demonstrate the effective plasma levels required to produce pain relief in a majority of patients (13 of 18, or 72%) is in the range of 50 to 150 ng/ml. (See Figure 4 at p. 7 of the Max 1991 article.) This translates to a total plasma concentration of 188 to 540 nM, or approximately 9 to 28 nM, if one were to correct for the portion of free drug available to associate with the receptor, knowing the other portion is bound to plasma proteins. Based on Figure 4 of the Max 1991 article, there is a reasonable probability that a significant portion (approximately 50%) of the H_1 and α_1 -adrenergic receptors would also be occupied at plasma concentrations that produce relief from neuropathic pain. Consequently, one cannot definitively conclude that desipramine produces relief from neuropathic pain by interacting solely at the norepinephrine transporter site.
10. The data reported in Tables I and II for (S,S) reboxetine stand in stark contrast to the corresponding data for desipramine. Specifically, (S,S) reboxetine exhibits surprisingly exceptional selectivity (>15,000) for the norepinephrine transporter over that of the serotonin transporter. See Table II. Consequently, and in contrast to desipramine, one can definitively conclude that compounds, such as (S,S) reboxetine, produce relief from chronic pain solely through their highly selective interaction with the norepinephrine transporter site.
11. Still further, the data reported in Table I conclusively shows that (S,S) reboxetine is a highly selective inhibitor of the norepinephrine transporter site having almost 25,000 fold selective response over other transporter/receptor sites (5-HT_{2A}, H_1 , α_1 -adrenergic, and muscarinic) believed to be responsible for adverse side effects. Such high selectivity is not exhibited by any of the comparative compounds. Thus, the

selectivity of compounds, such as (S,S) reboxetine, should provide an overall improved safety and tolerability far beyond that of conventional tricyclic antidepressants.

12. Values reported in Tables I and II for amitriptyline, desipramine, and fluoxetine were obtained from Table 3 in Owens *et al.* (1997) *J. Pharmacol. Exp. Ther.* **283**:1305-1322.
13. Inhibition constants reported in Tables I and II for (S,S) reboxetine and for racemic reboxetine at the norepinephrine and serotonin receptor sites were determined as follows:

Materials

- (a) All test compounds were obtained/made-available from commercial sources (Sigma, RBI) with the exception of reboxetine and its enantiomers, which were obtained from Pharmacia Research Compound Collection (Kalamazoo, MI). The following materials were used:
 - (i) [N-methyl-³H]-nisoxetine (purchased from Amersham Life Science (Buckinghamshire, England)); and,
 - (ii) [N-methyl-³H]-citalopram (purchased from Dupont New England Nuclear Products (Boston, MA)).

Binding Assays

- (b) Male Sprague Dawley rats were decapitated, and the cerebral cortical tissue was removed and homogenized in nine volumes of ice cold 0.32 M sucrose using a rotating pestle on 50 setting (10 up and down strokes).
- (c) The obtained homogenate was centrifuged at 1000 x g for 10 minutes at 4°C.
- (d) A supernatant was collected and centrifuged at 20,000 x g for 20 minutes at 4°C.
- (e) Following centrifugation, the protein pellet resulting from the centrifuging steps was re-suspended in a Krebs's Hepes Buffer pH-adjusted to 7.0, wherein the buffer contained 20 nM Hepes, 4.16 nM NaHCO₃, 0.44 nM

KH₂PO₄, 0.63 mM NaH₂PO₄, 127 mM NaCl, 5.36 mM KCl, 1.26 mM CaCl₂, and 0.98 mM MgCl₂.

- (f) Aliquots (5 ml each) were subsequently frozen and stored at -80°C; when needed, the aliquots were thawed at room temperature, and diluted to a final protein concentration of 30-150 µg of protein per well (or test tube) using the Krebs's Hepes Buffer.
- (g) Four-hundred microliter (µl) aliquots of homogenate and 50 µl aliquots of both the radioligand and the test compound were added to each test volume for a total volume of 500 µl.
- (h) The [N-methyl-³H]-nisoxetine binding assay was incubated for two hours at 25°C. The nonspecific binding was defined by using 10 µM desipramine.
- (i) The [N-methyl-³H]-citalopram binding assay was incubated for one hour at 25°C. The nonspecific binding was defined by using 100 µM fluoxetine.
- (j) The reactions were terminated by rapid vacuum filtration through Whatman GF/B glass-fiber filters (pre-soaked in buffer containing 0.5% polyethyleneimine for approximately four hours) mounted on a Brandel cell harvester (Model MP48).
- (k) The filters were washed rapidly three times using three milliliter aliquots of ice cold 0.9% saline.
- (l) The filters were subsequently assayed for radioactivity by liquid scintillation counting.

Uptake Assays

- (m) Uptake assays were performed using Madin-Darby canine kidney (MDCK) cell lines stably transfected with hNET, hDAT, or hSERT using conventional methods. See e.g., Wong *et al.* (2000) *Biol. Psychiatry* 47:818-29.
- (n) Cells plated in a 96-well harvester were washed with Krebs-Ringer-Hepes buffer two days following plating and preincubated at room temperature with test drugs for five to ten minutes prior to addition of radiolabeled substrates

([³H]dopamine for hNET and hDAT cells, [³H]serotonin for hSERT cells; all concentrations below 75 nM or 1/10 of respective substrate K_t values).

- (o) Incubation for ten minutes is terminated by removal of supernatant and two washes of the cells with the same buffer.
- (p) A scintillation cocktail was added to each well and the plates were counted using a Wallac MicroBeta scintillation counter at an efficiency of approximately 35%. Eleven to twelve concentrations of inhibitor (with two or four replicates) in the range of 100 pM to 1 mM were used in each assay.
- (q) Identical drug dilutions were used on any day to assay plates of hNET, hDAT, and hSERT MDCK cells in parallel.
- (r) Levels of nonspecific uptake are defined with 10 μM nomifensine (hNET and hDAT), 10 μM citalopram (hSERT), or with 100 μM cocaine.
- (s) Uptake velocities were normalized to control conditions in each experiment, and the obtained data were analyzed with GraphPad Prism 3.0a using the four parameter logistic equation in which the maximal value is allowed to float.

14. Except where otherwise noted in the Tables, the inhibition constants reported in Tables I and II for (S,S) reboxetine and for racemic reboxetine at the 5-HT_{2A}, H₁, α₁-adrenergic, and Muscarinic transporter/receptors sites were determined using standard protocols established by Cerep (Le Bois L'Eveque, BP1, 86 600 Celle l'Evescault, FRANCE), and the following radioligands:

- (a) [³H]-ketanserin for the 5-HT_{2A} site;
- (b) [³H]-pyrilamine for the H₁ site;
- (c) [³H]-prazosin for the α₁-adrenergic site; and,
- (d) [³H]-pirenzepine, [³H]-methoctamine, [³H]-4-DAMP for the respective M₁, M₂, and M₃-M₅ Muscarinic sites.

15. The techniques/assays set forth in the preceding paragraphs and in Owens *et al.* (1997) *J. Pharmacol. Exp. Ther.* **283**:1305-1322, to obtain the data reported in the

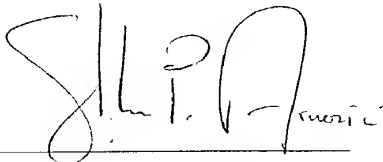
attached Tables I and II are standard receptor binding techniques and functional reuptake assays.

16. These techniques and assays are amenable to high throughput screening (HTS) technologies that use robotics to automate handling and dilutions of compounds, and can be performed by one skilled in the art of HTS to identify compounds with potential for pharmaceutical applications.
17. Although it was surprising and unexpected that (S,S) reboxetine exhibits remarkably greater selectivity for the norepinephrine transporter than it does for the serotonin transporter, it would be possible to search for such compounds using HTS technologies without undue experimentation.
18. Furthermore, it is entirely conceivable that up to 500,000 novel compounds could be screened within a three-month time period for their ability to selectively interact with the norepinephrine transporter.
 - (a) One such HTS screening process could be performed at a single concentration (1 μ M) and examine the ability of the screening compound to displace [N-methyl-³H]-nisoxetine, a relatively selective ligand for the norepinephrine transporter, from rat brain membrane preparations.
 - (b) Compounds found to be active would be confirmed by completing a full concentration-response curve, and the initial selectivity against the serotonin transporter would be confirmed using [N-methyl-³H]-citalopram, a relatively selective serotonin ligand.
 - (c) The functional nature of these interactions with human recombinant norepinephrine, serotonin, and DAT transporter sites could be confirmed in secondary screening assays.

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19. All statements made herein are of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under 18 U.S.C. § 1001 and may jeopardize the validity of the application or any patent which may issue thereon.

March 22, 2002



Stephen P. Arneric

TABLE I. Inhibition Constants (K_i , nM) of Compounds for Various Monoamine Transporter and Receptor Sites

Site	Norepinephrine	Serotonin	5-HT _{1A}	H ₁	α_1 -adrenergic	Muscarinic
(S,S) Reboxetine	0.21 \pm 0.03	>3000	>5000	>5000	>5000	>5000
Racemic Reboxetine	1.04 \pm 0.20	134 \pm 11.5	>1000	312 ^a	>5000 ^a	>5000 ^a
Amitriptyline ^a	8.6 \pm 0.04	16 \pm 0.8	5.3 \pm 0.2	0.17 \pm 0.01	4.4 \pm 0.2	2.6 \pm 0.1
Desipramine ^a	0.31 \pm 0.01	129 \pm 7	115 \pm 13	31 \pm 1	23 \pm 1	37 \pm 1
Fluoxetine ^a	473 \pm 11	2.0 \pm 0.1	141 \pm 9	933 \pm 23	1353 \pm 17	512 \pm 12

TABLE II. Selectivity for the Norepinephrine Transporter Over the Serotonin Transporter

Site	Norepinephrine (K _i , nM)	Serotonin (K _i , nM)	Selectivity ^c for Serotonin (K _i)/Norepinephrine (K _i)
(+)-[S,S]-Reboxetine	0.21 \pm 0.03	>3000	>15,000
Racemic Reboxetine	1.04 \pm 0.20	134 \pm 11.5	129
Amitriptyline ^b	8.6 \pm 0.04	16 \pm 0.8	1.8
Desipramine ^b	0.3 \pm 0.01	129 \pm 7	430
Fluoxetine ^b	473 \pm 11	2 \pm 0.1	0.004

^a Values were obtained from Table 19-3 in R.J. Baldessarini, Depression and Mania, Goodman & Gilman's The Pharmacological Basis of Therapeutics, 10th Ed., Chapter 19, pp. 447-483, (2001).

^b Values were obtained from Table 3 in Owens *et al.* (1997) *J. Pharmacol. Exp. Ther.* 283:1305-1322.

^c Unit-less values were calculated by dividing the K_i value for the serotonin transporter site by the K_i value for the norepinephrine transporter site.